Developing an *in vitro* myobundle model system for pathophysiological studies of human skeletal muscle

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Background

- Existing *in vitro* models for liver, lung, and cardiac tissue have made recent notable progress, however a predictive model of human skeletal muscle does not exist
- There are many metabolic, neuromuscular, and dystrophic disorders of the skeletal muscle that currently lack therapies
- Disease of the skeletal muscle has high societal impact including diabetes, obesity, and various dystrophies
- Skeletal muscle involved in interactions between organs has been important in cognition, aging, inflammation, and cancer
- Two-dimensional cultures of myoblasts are well known but these cultures lack the organization and function of a native muscle and are hard to maintain over time
- The usefulness of these existing models in pharmacological studies and disease modeling is limited

Purpose

- To adapt a novel, physiologically relevant, three-dimensional skeletal muscle model and confirm that it is usable in future studies
- To fabricate myobundles which contract spontaneously in response to electrical and chemical stimulation
- To develop a tool for pharmacological testing and disease modeling
- To provide an alternative to costly animal studies, mitigating ethical considerations and reducing the number of *in vivo* and *ex vivo* models

Materials

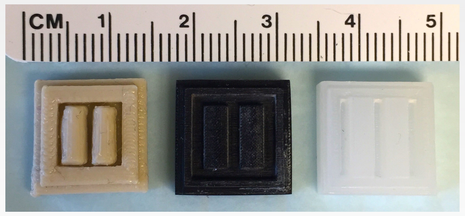

Figure 1. Negative molds were used to generate multiple replicas of the positive PDMS molds. Three prototypes of negative molds are pictured above. From left to right: (left) 3D printed polylactic acid (PLA) plastic, (middle) initial laser cut acrylic plastic with incorrect dimensions, (right) current model of laser cut acrylic plastic with the correct dimensions.

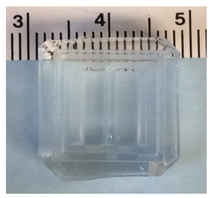

Figure 2. Positive molds were cast from negative molds using Sylgard 184 Elastomer cured for 24 hours at 64°C.

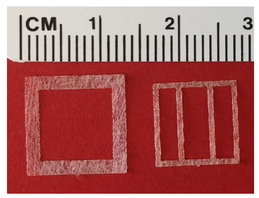

Figure 3. Laser-cut Cerex teflon frames fit into the outer ridge of the PDMS molds.

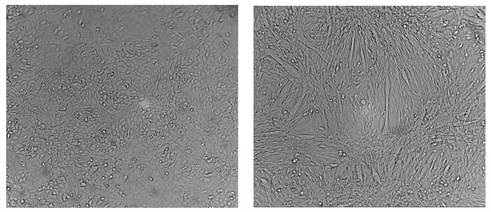

Figure 4. C2C12 cells, mouse myoblast cell line (left), were cultured in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum and then differentiated to form multinucleated myotubes (right).

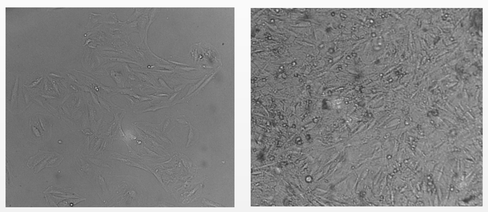

Figure 5. H9C2 cells, a subclonal line derived from embryonic rat heart tissue. Myoblasts (left) were cultured in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum and then differentiated to form multinucleated myotubes (right).

Methods

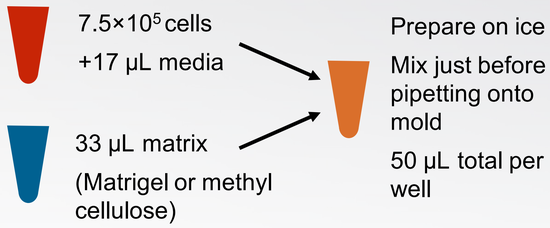

Figure 6. Expanded myoblasts were dissociated in TrypLE, pelleted, and resuspended to give a working concentration of 15×10^6 cells/mL.

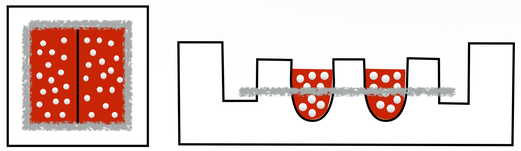

Figure 7. The graphic on the left is a representation of cells and matrix viewed from above. The graphic on the right is a side view of matrix and cells seeded in the positive mold. The grey line is representative of the frame which is in contact with the cell suspension.

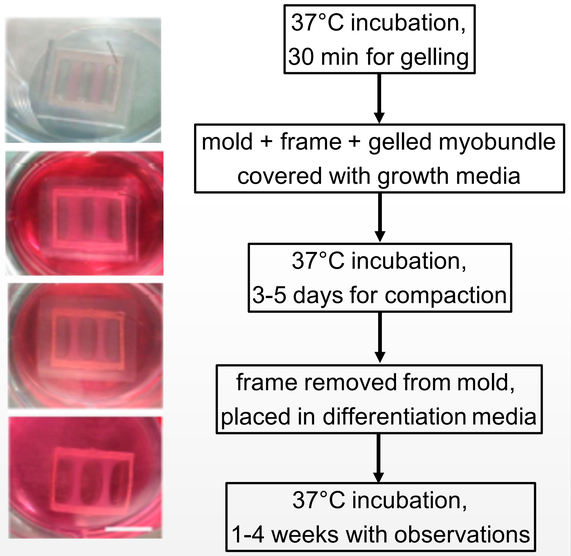

Figure 8. Myobundle fabrication procedure following initial plating of mold. Images (Madden et al.).

Results

- Attachment to the frame has been observed but cell death has soon followed
- Our observations do prove, however, that formation of the myobundle is possible with this model and that with continued alteration the myobundle should survive for use in further testing

Future Directions

- Proceed to vary the cell type and concentration in order to find cells for optimal growth in the three-dimensional matrix
- Use micro patterned PDMS silicon to orient myotubes for growth and differentiation
- Histological staining to confirm mature myobundles
- Measure the contractile and biochemical responses of myobundles to determine their usability in preclinical testing and disease modeling
- Use human iPSCs to allow for production of human muscle cells and also allow generation of patient specific cells that can harbor mutations leading to disease states, such as muscular dystrophy
- Study the effects of the TRIM protein MG53 on membrane resealing

References

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